CONTRIBUTIONS TO THE HISTORY OF THE DEVELOPMENT OF THE PYRENOMYCETES.*

(Plate IX.)

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The *Ascomycetes* have already been the object of many researches resulting in a series of most interesting facts which in turn always suggest new questions. To solve these requires investigations in two directions. First, the development and significance of the separate organs, especially of the pycnidia and perithecia, should be better established. Athough much has been done in this respect, still the manifold forms of the *Ascomycetes* leave many new facts to be expected, and the results already obtained are too scattered to admit of any generalization. Second, the life history of the pleomorphic forms demands thorough investigation. We know a large number of pycnidia and other gonidia forms, and conclude that each represents a stage in the development of an Ascomycete, but this has been definitely proved in only a very few cases.

What follows contains a series of observations in the two directions already indicated, having as their object some gonidia forms and *Pyrenomycetes* of doubtful relationship, but not giving a complete chain of development. We will first attempt to solve the question as to what cycle of development the common *Glœosporium nervisequum*, (Fckl.) Sacc., a dangerous enemy to the sycamore trees, belongs. For reasons given below the question must remain almost entirely unanswered, but other forms of fungi were found in the course of these investigations. A pycnidium, *Discula platani*, (Pk.) Sacc., is suspected of belonging to the same cycle of development as the *Glœosporium* and was consequently very closely investigated. Together with this *Discula* appeared a *Fenestella* whose life history could only be established to a certain degree of completion. A *Cucurbitaria* found growing with the two other forms was studied in relation to the development of its pycnidium, more exhaustive observations being prevented by the lack of material. In what follows I have brought together my observations on these four forms.

It should be mentioned that the three last-named fungi live on dry branches. It is well known that these, like lichens that grow upon bark, can not be cultivated except with difficulty. Hitherto they have only attained a limited age in culture fluids. It is extremely difficult to cultivate them on dry twigs because it is impossible to sterilize other twigs without destroying the objects of our observations, and foreign fungi, which make observations difficult and results uncertain, invade the cultures and in many cases so get the upper hand as to destroy all other fungi. *Tricothecium roseum*, Lk., is an especially dangerous fungus.

* *Bot. Zeitung*, 1886, p. 824.

It is with difficulty that a twig can be kept in a moist chamber without this species making its appearance.

I. Glœosporium Nervisequum, (Fckl.) Sacc.

The *Platanus* (sycamore) frequently exhibits an epidemic disease that is especially injurious to the young trees. The first manifestation of the malady is the wilting of the young leaves. Soon after they unfold, about the middle of May, brown spots make their appearance on any portion, and extend along a vein, toward the base, over the leaf, and even on the pedicel, until the leaf finally falls off. Upon these dry places are small black dots just visible to the naked eye, and representing the gonidial form of a fungus probably belonging to the *Pyrenomycetes*. It has been seen in most cases on *Platanus occidentalis*.* Léveillé, Fuckel, and Saccardo state that it also occurs on *Platanus orientalis*, but I have not been able to prove these statements. In addition to this, Fuckel refers to a form growing on the oak,† which he has distributed in his F. Rhen. No. 428.

This fungus has been known for a long time. It was first described by Léveillé as *Hymenula platani* in 1848, but it is surprising that neither he, Fuckel, or Saccardo mention its destructiveness. In his *Symbolæ*, p. 369, Fuckel cites it as *Fusarium nervisequum* and gives an illustration of one of the spores. In the F. Rhen. No. 427 it is called *Labrella? nervisequum*, Fckl. Saccardo placed the fungus in the broader genus *Glœosporium* and adopted Fuckel's specific name, since a *Glœosporium platani*, (Mont.) already existed. It is therefore known as *Glœosporium nervisequum*, (Fckl.) Sacc.

If the infected areas are placed under a low magnifying power they show brown or black pustules which are elongated in form and most generally located in the angle formed by the vein and the leaf surface, but are also found on both these parts. Generally they occur upon the upper side of the leaf; much more rarely upon the lower.

The structure of the fungus may be seen in a cross-section of one of these pustules (Fig. 1). The fungus destroys the walls between the epidermal cells, and the outer wall with the cuticle arches up until it bursts when the spores are ripe. The base of the pustule is lined with a pseudo-parenchymatic tissue composed of small cells which may be called a stroma. From this arise numerous hyphæ which penetrate the leaf, passing between the cells and completely destroying the leaf tissues. From the upper side of the stroma numerous hyphæ or basidia grow out into the cavity of the pustule. These swell up and become club-shaped and cut off spores from the end. They are of unequal lengths, and the club-shaped swellings begin with the growth of the basidia. The spores are detached in great numbers; when the epidermis ruptures they exude in the form of a worm-like, whitish-yellow mass. They are very irregular in shape, being mostly elliptical or pear-shaped, and

* It has been found in California on *P. orientalis* by Harkness.

† Specimens on oak have been sent to the Department from Indiana.

are always one-celled and colorless (Fig. 2). The smooth, delicate spore membrane is surrounded by a gelatinous envelope. The spores measure 9–14 by 5–6μ, agreeing with the measurements made by Fuckel and Saccardo. They germinate in a few hours in water or a nutrient solution, and develop a germ tube which soon branches and forms septa; the cells lying next to the spore swell more or less.

A mycelium is formed in a few days by means of the rapid growth of the germ tube and repeated branching. The hyphæ are of unequal diameter, and are made up of short, often somewhat swollen cells. When the mycelium grows in a nutrient solution gonidia are formed after some time. The short cells of the thicker hyphæ develop outgrowths which are cut off, the mother cell and gonidia both being filled with dense protoplasm; or on the other hand, the hyphæ cells may first develop basidia, which cut off spores from the end. Both forms of development may occur simultaneously on the same hyphæ. The gonidia themselves are alike, and agree in form and size with those produced on the leaf excepting that they are a little more regular. From this, and from the fact that the gonidia are produced more abundantly where the hyphæ are more closely interwoven, we may conclude that the gonidia produced in culture on the slide are homologous with those produced on the leaf, and that the hymenium not attaining to the same degree of development is due to the changed mode of growth. This question can not be definitely decided, for the fungus on the slide did not develop farther, and attempts at infection were without result.

Leaves of *Platanus* were infected with the *Glœosporium* in the most varied ways—upon the upper and lower surface, upon young and old leaves, on detached branches and uninjured trees; the cultures were kept moist and dry, and the germinating power of the spores was controlled by cultures on slides, but not a single infection gave a positive result. It can therefore at present only be said in regard to the life history of the *Glœosporium nervisequum* that the gonidia will produce a similar gonidial stage on the slide.

Is it possible, then, to draw the conclusion that this closes the life history of the fungus; that is, that it has lost the perithecia and pycnidia stages, as has been supposed true of *Botrytis bassii*, *Isaria strigosa*, and *Oidium lactis*, although we are by no means forced to such a conclusion? If this is true, we must assume that the spores fall to the ground, survive the winter among the fallen leaves, and in the spring are carried to the leaves by means of the wind or some other agent. This inference is supported by the fact that the disease begins nearest the ground. The leaves on the lower branches wither first; the upper ones gradually follow. On the other hand, it is not conceivable that these delicate, thin-walled spores could survive the winter lying upon the damp earth, especially since they germinate very readily on the slide upon the addition of moisture without requiring a resting period. And if these were the agents in the penetration of the leaves it is not likely that all

our experiments would meet with negative results. The evidence rather points towards discarding the idea that *Glœosoporium nervisequum* has no other stages in its life history.

It has not yet been possible to find out these other stages; the cultures could not be carried far enough, and forms that might belong to the *Glœosporium* were found in the open air on fallen leaves and dry branches, but we were not able to establish the connection. One form which we especially suspected of organic relationship, on account of its morphological evidence, is described in detail in what follows.

II. Discula platani, (Pk.) Sacc.

Upon *Platanus* trees attacked by *Glœosporium* there are many dry branches of the previous year's growth, which died after all the leaves had fallen, and before the close of the vegetative period. Upon these are usually a large number of small pustules which finally split open. At first they look very much like lenticels, and can scarcely be distinguished from them by the naked eye; but when one of these twigs is moistened small yellowish columns emerge from all the openings; these columns are composed of spores, and show the presence of a fungus.

The structure of the fungus may easily be studied in a cross-section of the branch. The young pustules are filled with a pseudo-parenchymatic tissue which rests upon the green part of the bark and penetrates it slightly. Above (by above is meant the side turned away from the twig) the fungus pushes up the bark until it finally bursts (Fig. 3). The tissue is then in the form of a cone. The basal cells are nearly isodiametric and polygonal, but the upper ones are elongated and lie more or less parallel. The point of the cone does not project beyond the bark, and the hyphæ are more apt to swell up when they reach the surface. The entire cone shows a strong upward growth which has its origin in the lower cell layer. This layer has here the function of meristem and pushes up other portions of the cone by the elongation of its cells.

In more advanced stages pseudo-parenchyma is also developed in the uppermost layer of bark parenchyma whose cells have been completely destroyed. A cavity quickly arises in the cone by the cessation of growth in the central part (Fig. 4). Hyphæ very quickly grow out into the cavity from all sides and clothe it with a hymenium which produces spores by successive abscision. The cavity enlarges especially towards the surface; the remains of the parenchyma together with the cone above them are pushed up considerably, enlarging the slit in the bark. The hyphæ forming the cone generally become completely obliterated; very rarely only a pore is formed therein; by these processes the hymenium is exposed. The outer portions become dark colored and the entire pustule is more or less bowl-shaped (Fig. 5).

The basidia are unbranched, slender, and cylindrical. The spores are

one-celled, colorless, oval to pyriform, surrounded by a thin gelatinous envelope, and measure 10–14 by 5–7μ. They have a striking resemblance to the spores of *Glœosporium nervisequum*.

It is evident from the form of the open conceptacle that the fungus belongs to the *Excipulaceæ*. It agrees perfectly with the description of *Discula platani* Peck (Sacc. Syll., Vol. III, p. 694).

On account of its mode of development *Discula platani* must be regarded as a pycnidium, but it differs in several points from what we usually call pycnidia. The body of tissue remains intact longer than is usual and it is also different in being differentiated into two kinds of tissue. The upper part of the pycnidium with the elongated cells corresponds with similar formations on the walls of other pycnidia, as will be shown below in case of *Fenestella* (Fig. 11). The pore always makes its appearance at the spot where the elongated cells were developed. In *Discula* this attains such very considerable dimensions that the entire pycnidium is destroyed down to the bowl-shaped basal portion, and besides this the phenomena of growth are peculiar to *Discula*. *Discula* does not, however, stand alone in this. Banke (Beitr. z. Kenntn. d. Pycniden in Nova Acta Acad., Leopold., XXXVIII, p. 481) demonstrated an apical growth of the pycnidia of *Pleospora polytricha*.

Nothing is known concerning the farther development of the *Discula*. In fact it was only in rare cases that we succeeded in following the development until the hymenium was exposed. A few days after the branches were brought into the house *Tricothecium roseum* attacked the pycnidia where they had broken through the bark and completely destroyed them.

When sowed in water or nutritive fluid the spores of *Discula* germinate in about twenty-four hours. They generally give rise to two, more rarely one or three, germ tubes, which swell up, forming spherical bodies. The growth is at first similar to the budding that occurs in yeast, showing repeated branching and budding, surrounding the spore in a dense tangle. One of the branches finally exhibits a decided apical growth and develops into a hypha branching monopodially. No septa are present for some time, but they appear later in considerable numbers. A vigorous mycelium develops rapidly but it never attains the production of spores. Frequently a few cells either at the ends or along the course of the hyphæ swell up and assume a spherical shape, and sometimes this happens for two adjacent cells; in the latter case the sphere is divided by a septum. The contents is watery and the protoplasm simply forms a lining within the wall.

Leaves and branches of *Platanus* were inoculated with *Discula* in the same manner as with the *Glœosporium* but with no results. The leaves remained fresh for a long time and then began to wither and turn brown without showing any signs of a fungus.

Discula platani is found on dry branches of *Platanus* during the entire year, and it was once discovered upon the petiole of a large decaying

leaf, in December. Owing to the fact that it is always found associated with *Glœosporium nervisequum* and in its immediate vicinity, it is suspected that the two are stages in the development of the same fungus, the more so because the spores are so very similar. It is conceivable that the mycelium passes from the petioles into the branches and there produces the pycnidia of *Discula* whose spores develop into *Glœosporium* upon the leaves.* But such a connection could not be established either by natural or artificial methods, and the question still remains an open one.

(*To be continued.*)

NORTH AMERICAN AGARICS.

(Genus *Russula* (*russulus*, reddish). Fr. Hym. Eur., p. 439.)

By ROBERT K. MACADAM.

PART I.

Pileus fleshy, convex then expanded, and at length depressed; stem *stout*, polished, *not corticate*, generally *spongy* within, confluent with the hymenophore; gills *nearly equal*, *milkless*, *rigid*, *brittle*, with an acute edge, sometimes dropping *water;* trama vesiculose; veil entirely *obsolete;* spores white or very pale yellow, generally echinulate.

Habitat.—On the ground, generally in woods or the vicinity of trees in summer and autumn.

This genus is interesting on account of the beauty and brilliant coloring of many of its species, and especially so to amateurs, as it is one of the few divisions of *Agaricini* which can be readily distinguished. Members of it may be recognized by the stout spongy stem, dry texture, and extreme brittleness; they are generally found in grassy woods and are of nearly all colors, frequently with the cap a brilliant red, pure white, or white blotched or shaded with red. *Russula* is allied to *Lactarius*, but is distinctly separated by the absence of *milk* in the gills; those of some *Russulæ* distill drops of water, especially in rainy weather. The internal structure is also related, as shown by the presence, in the acrid species, of the milk-secreting vessels of *Lactarius*, but in an undeveloped form.

* At the bases of the infected shoots this spring there was almost invariably a dead area on the lignified branch, and mycelium was invariably present in the tissues; this mycelium penetrated into the vessels of the wood and could not be morphologically distinguished from that in leaves infested with *G. nervisequum*. Many buds had died either in late autumn or during winter and there were similar but larger dead areas around them, and in these *Discula platani* often made its appearance. Indeed, it is almost impossible to avoid the conclusion that the mycelium of *Glœosporium nervisequum* extends into the woody parts of the branches, where the fruit of the fungus assumes a different form. The formation of the mass of pseudo-parenchyma may possibly be explained on the ground that it is necessary in order to rupture the epidermis and cork layer of the bark; and when this is accomplished it disappears.—E. A. S.